

Counter-current liquid carbon dioxide purification of a model reaction mixture

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Abstract

The enzymatic reaction of soybean oil (SBO) triacylglycerides (TAGs) with ethyl ferulate (EF) produces the product feruloylated acylglycerols, called SoyScreen™ as well as by-product fatty acid ethyl esters (FAEEs). Both the unreacted EF as well as the by-product FAEEs must be removed from the mixture before the feruloylated acylglycerols can be used as natural sunscreens and/or antioxidants. This research investigated the use of a continuous liquid carbon dioxide (L-CO₂) counter-current fractionation method to purify a model mixture representing the crude enzymatic mixture from the synthesis of SoyScreen™. The effect of column length, solvent to feed ratio (S:FR) and feed rate were examined. Raffinate purity increased with both column length and S:FR. When the feed rate was doubled while the S:FR was held constant, there was essentially no loss of raffinate purity. Using L-CO₂ (23–25 °C and 11.0 MPa), a 120 cm long packed column, a S:FR of ca. 11.9, the raffinate was essentially free of both EF and FAEEs at feed rates of either 0.464 or 0.927 g/min with ca. 90% yield.

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1. Introduction

An enzymatic transesterification of soybean oil (SBO) triacylglycerides (TAGs) with ethyl ferulate (EF) to make feruloylated acylglycerols (FAGs), called SoyScreen™ has been previously described [1–3] and patented [4] with structures of the starting materials and products provided [3–4]. These FAGs are believed to have great potential as both natural sunscreens, and as antioxidants for both the cosmetic and the food industry [5]. The enzymatic synthesis of SoyScreen™ is currently being scaled-up for commercialization of this product at the National Center for Agricultural Utilization Research. However, recent attempts to purify the SoyScreen™ product using molecular distillation have failed to completely remove all the residual starting material, ethyl ferulate (EF) from the crude reaction mixture. In addition, there is an adverse change in the color of the product during the molecular distillation. Therefore, an improved purification method for the reaction mixture

is needed to help commercialize this product. Earlier research has demonstrated that both supercritical carbon dioxide [6] as well as liquid carbon dioxide (L-CO₂) [7] can be used to separate hexane from SBO, giving SBO with extremely low levels of residual hexane. In addition, it has previously been shown that L-CO₂ can effectively remove both EF as well as by-product fatty acid ethyl esters (FAEEs) from the reaction mixture to purify SoyScreen™, although this previous research only involved batch processing of relatively small amounts of material [3]. The benefits of continuous counter-current processes, including those utilizing supercritical fluids have been described previously [8]. Examples of this technique include the supercritical carbon dioxide (SC-CO₂) counter-current separation of squalene and methyl oleate from a model mixture [9] and the purification of tocopherols [10,11] and squalene [12] from deodorizer distillates (DD). In addition, SC-CO₂ counter-current fractionation has been used to deacidify rice bran oil [13] and remove ethanol from brandy [14]. The objective of this research was to investigate the use of a continuous L-CO₂ counter-current fractionation method as a means to purify a model mixture representing the crude enzymatic mixture from the synthesis of SoyScreen™.

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2. Experimental

2.1. Model matrix feed

A solution of EF, FAEE and SBO TAGs with a molar ratio of 1:1:2 (EF:FAEE:TAGs) representing the product mixture from the enzymatic reaction of EF and TAGs was prepared as a model matrix mixture to study the separation of the various components using counter-current L-CO₂ fractionation. Although the crude reaction mixture from the enzymatic synthesis of SoyScreen™ is actually ca. 1:1:1:1 (EF:FAEE:TAG:FAG), there were insufficient quantities of this mixture to conduct the fractionation studies. In order to determine the feasibility of using L-CO₂ to perform the purification of the actual crude SoyScreen™ mixture and investigate process variables, the model matrix was used instead. In the model matrix, one equivalent of TAG was substituted for one equivalent of FAG giving a total of two equivalents of TAGs. The molecular structures of TAGs and FAGs are very similar with the substitution of one ferulic acid moiety for one of three fatty acid moieties on half the glyceride backbones being the only difference between the mixtures. The EF:FAEE:TAG model mixture was expected to behave almost identically to the EF:FAEE:TAG:FAG mixture. During previous L-CO₂ separations of FAEEs and EF from the crude SoyScreen™ mixture [3], it was noted that FAEEs were extracted more readily than EF, even though EF has a lower molecular weight (i.e., 222) than a typical FAEE from SBO such as ethyl linoleate (EL) (i.e., 308). This is because EL is less polar and liquid at room temperature, while EF is solid at room temperature. The lower polarity and higher vapor pressure of EL make it more soluble in L-CO₂ than EF. Therefore, the replacement of a fatty acid by ferulic acid on a TAG should result in a lower solubility of the FAG relative to the corresponding TAG. The lower solubility of the FAG in L-CO₂ should actually make the separation of the FAEEs and EF from FAGs even easier than the separation of the FAEEs and EF from TAGs.

The model matrix mixture was warmed to 60 °C to dissolve all of the components and drawn into an ISCO model 260D syringe pump (ISCO Inc., Lincoln, NE) also held at 60 °C. This solution was then pumped into the top of the fractionation column and flowed down through the column. The density of this solution was determined to be 0.927 g/mL using a mini weight per gallon cup (P.N. Gardner Co. Inc., Pompano Beach, FL, USA).

2.2. Fractionation column

A schematic of the stainless steel column used for the L-CO₂ counter-current fractionations is shown in Fig. 1. The column has an internal diameter of 1.75 cm and was packed with protruded stainless-steel packing (0.41 cm Pro-Pak) (Cannon Instrument Co., State College, PA, USA) with a 94% void volume. Two column heights were tested (60 and 120 cm) with internal volumes of ca. 144 and 288 mL, respectively.

All fractionations were done at room temperature (i.e., ca. 23–25 °C) and 11.0 MPa. Previous research [3] demonstrated that the combination of 25 °C and 11.0 MPa was very effective

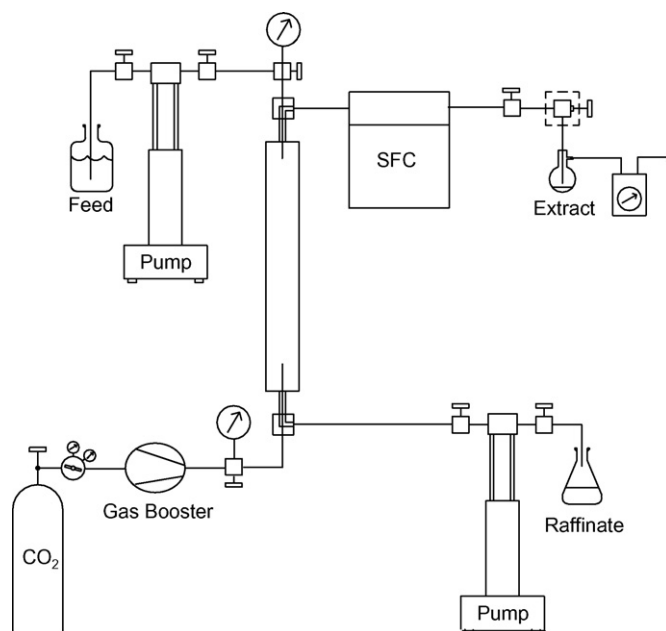


Fig. 1. Schematic of liquid carbon dioxide counter-current fractionation system.

in a similar batch purification process. In addition, a relatively low pressure (i.e., 11.0 MPa) and a temperature near ambient (i.e., 23–25 °C) were chosen specifically to avoid higher costs due to higher capital costs and energy inputs associated with separations done at elevated pressures and temperatures.

Welding-grade CO₂ (i.e., solvent) (Airgas Inc., Radnor, PA, USA) was fed from a cylinder to a Haskel model AG-30-C gas booster pump (Haskel International Inc., Burbank, CA, USA).

The column was pressurized to 11 MPa and equilibrated for 10 min before the extraction commenced. Liquid CO₂ entered the bottom of the column, moved up through and out the top of the column, through the injector of a supercritical fluid chromatograph (SFC), across a micro-metering valve and into a tared 100-mL round bottom flask (to collect the extracted components), and the expanded CO₂ exited at a flow rate of ca. 3 or 6 L/min (STP) through a dry gas test meter (model DTM-200A, American Meter Corp., Philadelphia, PA, USA) before it was vented to the atmosphere. Depending on column length and feed rate, approximately 300–600 L CO₂ (STP) were used to perform each extraction.

An in-line supercritical fluid chromatograph (SFC) was used to monitor the composition of the extract during the course of the fractionation and to determine when a steady state was achieved and was similar to a system described previously [15]. A stable extract composition in the L-CO₂ was generally achieved within 100 L expanded CO₂ (STP). The in-line SFC analyses of the components in L-CO₂ were conducted with a Lee Scientific Series 600 SFC (Dionex Corp., Salt Lake City, UT, USA) equipped with a flame ionization detector held at 350 °C. SFC/SFE-grade carbon dioxide (Airgas Inc.) was used as the carrier fluid. A SB-Octyl-50 capillary column (10 m × 50 μm i.d., 0.25 μm film thickness) (Dionex Corp.) was used. The program used was: 100 °C isothermal, 13.2 MPa for 3 min, and then 2 MPa/min to 29.4 MPa. Injections were made as timed-

Table 1
Treatment descriptions for L-CO₂ counter-current fractionation

Treatment number	Column length (cm)	Feed rate (g/min)	Solvent (CO ₂) rate (g/min)	Solvent:feed ratio (g/g)
1	60	0.116	5.50	47.4
2	60	0.232	5.50	23.7
3	60	0.464	5.50	11.9
4	60	0.927	5.50	5.9
5	60	0.464	10.99	23.7
6	120	0.232	5.50	23.7
7	120	0.464	5.50	11.9
8	120	0.927	5.50	5.9
9	120	1.854	5.50	3.0
10	120	0.927	10.99	11.9

split injections with a Valco valve for 0.5 s with a 200 nL loop. Chromatographic data was quantified using a Data-Jet integrator (Spectra Physics Inc., Fremont, CA, USA).

The extracted model matrix (i.e., the raffinate) exited the bottom of the column and was drawn into a second ISCO model 260D (ISCO Inc.). The flow rate of the feed pump and the flow rate of the raffinate were matched to maintain the pressure within the fractionation column. After each extraction was complete, the raffinate was pumped out of the ISCO pump into a collection bottle for analysis.

2.3. Experimental treatments

The experimental conditions used for the various treatments tested are described in Table 1. Two different column lengths were tested (60 cm versus 120 cm) as well as five solvent to feed ratios (S:FR) (3.0, 5.9, 11.9, 23.7 and 47.4 g/g). The effect of feed rate independent of S:FR was also examined. Using the 60 cm column, the feed rate was doubled from 0.232 g/min (i.e., treatment 2) to 0.464 g/min (i.e., treatment 5) while holding the S:FR constant at 23.7. In addition, using the 120 cm column, the feed rate was doubled from 0.464 g/min (i.e., treatment 7) to 0.927 g/min (i.e., treatment 10) while holding the S:FR constant at 11.9. Each experimental treatment was replicated twice.

2.4. Compositional analyses

At the end of the fractionation, the compositions of both the extract as well as the raffinate were determined by off-line supercritical fluid chromatography. The SFC analyses of the components in L-CO₂ were conducted with a Series 4000 SFC (Selerity Technologies, Inc., Salt Lake City, UT) equipped with a flame ionization detector (FID) held at 350 °C. SFC/SFE-grade carbon dioxide (Airgas Inc.) was used as the carrier fluid. A 3B-Methyl-100 capillary column (10 m × 50 μm i.d., 0.25 μm film thickness) (Selerity Technologies, Inc.) was used. The program used was: 100 °C isothermal, 10.1 MPa for 5 min, and then 1.5 MPa/min to 31.4 MPa. One microliter of a solution containing ca. 5 mg/mL was injected into the SFC and the relative amounts determined from the FID area percents. A single SFC analysis was performed on each sample.

Analyses of variance (ANOVA) were conducted on both extract as well as raffinate composition percentage data (i.e., TAG, EF and FAEE) using Statistix 7 software (Analytical Software, Tallahassee, FL, USA) and means were compared using least significant difference (LSD). The main effects of column length and S:FR were tested using *F*-tests. Mathematical equations that described the data were determined using TableCurve™ 2D curve-fitting software (Systat Software, Inc., Richmond, CA, USA).

3. Results and discussion

The FID area percentages of TAGs in the raffinate fractionated by the L-CO₂ are shown in Fig. 2. The ANOVA indicated that there were highly significant effects of both column length ($F_{1,14} = 62.7$, $P = 0.0000$) as well as S:FR ($F_{4,14} = 144.3$, $P = 0.0000$). The 120 cm column gave a raffinate with a higher percentage of TAGs than did the 60 cm column. In addition, the percentage TAGs in the raffinate increased with S:FR for both column lengths. With the 120 cm column and a S:FR of ca. 11.9 (2.47 as ln), the raffinate was ca. 100% TAGs and essentially purified of both EF and FAEEs. However, with the 60 cm column, it required a S:FR of ca. 47.4 (3.86 as ln) to achieve a similar level of purity. Earlier research demonstrated an increase in squalene content in the raffinate with an increase in column length as well [9].

With the 60 cm column, when the feed rate was doubled from 0.232 g/min (i.e., treatment 2) to 0.464 g/min (i.e., treatment 5) while the S:FR was held constant at 23.7 (3.17 as ln), the percentage TAGs in the raffinate were statistically equivalent (98.9% and 98.5%, respectively). Similarly, with the 120 cm column, when the feed rate was doubled from 0.464 g/min (i.e., treatment 7) to 0.927 g/min (i.e., treatment 10) while the S:FR was held constant at 11.9 (2.47 as ln), the percentage TAGs in the raffinate were also statistically equivalent (99.9% and 99.3%, respectively). Therefore, total throughput could be doubled without significantly decreasing the purity of the raffinate. Although higher flow rates were not tested, it may be possible to increase the total throughput even further without adversely affecting the purity of the raffinate.

The FID area percentages of FAEEs and EF in the raffinate fractionated by the L-CO₂ are shown in Fig. 3. The ANOVA indi-

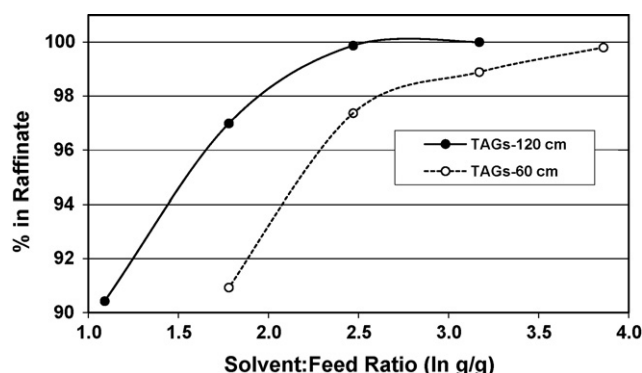


Fig. 2. Percentage TAGs in the raffinate as a function of column length and solvent:feed ratio.

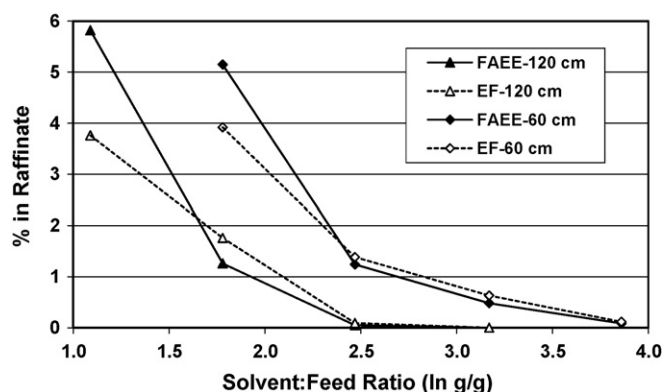


Fig. 3. Percentage FAEs and EF in the raffinate as a function of column length and solvent:feed ratio.

cated that there were highly significant effects of both column length ($F_{1,14} = 43.9$, $P = 0.0000$) as well as S:FR ($F_{4,14} = 110.7$, $P = 0.0000$) on percentage of FAEs. The 120 cm column produced a raffinate with a lower percentage of FAEs than did the 60 cm column. The percentage FAEs in the raffinate decreased as the S:FR increased for both column lengths. With the 120 cm column and a S:FR of ca. 11.9 (2.47 as ln), the raffinate contained almost no FAEs while it required a S:FR of ca. 47.4 (3.86 as ln) to achieve a similar level of purity with the 60 cm column. When the flow was doubled while the S:FR was held constant, the percentage FAEs in the raffinate were statistically equivalent with both the 60 cm column (treatments 2 and 5, 0.5% and 0.8%, respectively) and the 120 cm column (treatments 7 and 10, 0.1% and 0.4%, respectively).

The percentage EF in the raffinate followed the same pattern as that of the FAEs (Fig. 3). There were highly significant effects of both column length ($F_{1,14} = 101.2$, $P = 0.0000$) as well as S:FR ($F_{4,14} = 209.1$, $P = 0.0000$) on percentage of EF. The 120 cm column produced a raffinate with a much lower percentage of EF than did the 60 cm column and the percentage EF in the raffinate decreased as the S:FR increased for both column lengths. When the flow was doubled while the S:FR was held constant, the percentages of EF in the raffinate were statistically equivalent with both the 60 cm column (treatments 2 and 5, 0.6% and 0.6%, respectively) and the 120 cm column (treatments 7 and 10, 0.1% and 0.1%, respectively).

The FID area percentages of the components (i.e., TAGs, FAEs and EF) in the extract separated from the model matrix mixture by the L- CO_2 are shown in Fig. 4. The ANOVAs indicated that column length did not have a significant effect on the percentage of TAGs ($F_{1,14} = 2.31$, $P = 0.15$), FAEs ($F_{1,14} = 3.49$, $P = 0.08$) or EF ($F_{1,14} = 0.01$, $P = 0.94$) in the extract. However, the ANOVAs did indicate that S:FR had a highly significant effect on percentage of TAGs ($F_{4,14} = 62.9$, $P = 0.0000$), FAEs ($F_{4,14} = 106.8$, $P = 0.0000$) and EF ($F_{4,14} = 50.4$, $P = 0.0000$) in the extract. Because the ANOVA indicated that column length did not have a significant effect, the data presented in Fig. 4 represent the combined data for the two column lengths. For the EF, the percentage did not vary much with S:FR, and although there was a slight trend towards increasing percentage with S:FR, it decreased slightly

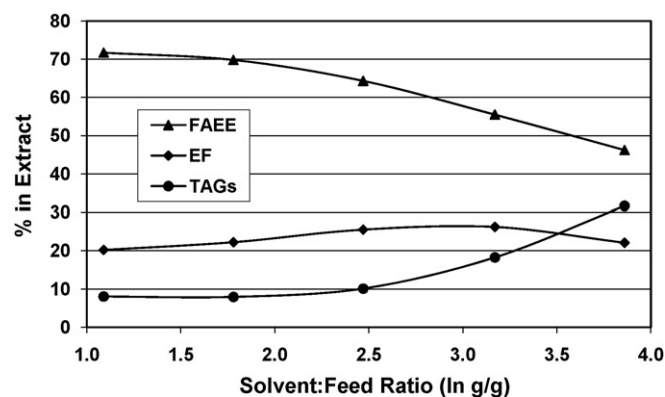


Fig. 4. Percentage FAEs, EF and TAGs in the extract as a function of solvent:feed ratio.

at the highest S:FR (i.e., 47.4, 3.86 as ln). The percentage FAEs in the extract decreased as the S:FR increased. Conversely, the percentage TAGs increased with S:FR. The percentage FAEs and percentage TAGs are clearly inversely proportional and this is also demonstrated by the highly significant negative correlation between these percentages ($r = -0.96$, $P = 0.0000$, $n = 20$). This suggests that the L- CO_2 has a limited solvating capacity for these two compounds and the presence of one limits the presence of the other.

Using L- CO_2 (23–25 °C) at 11.0 MPa, a S:FR of 11.9, with the 120 cm column, the raffinate was essentially 100% TAGs (i.e., pure) with no FAEs or EF remaining. In this case, the extract separated from the model matrix contained ca. 65% FAEs, 25% EF and ca. 10% TAGs, and the raffinate yield was ca. 90% of the TAGs in the original feed. Previous research has shown that SC- CO_2 at 11.5 MPa, a S:FR of ca. 20, with a 250 cm column, could purify squalene from 40% to ca. 95% with a yield of ca. 90% [9]. Similarly, SC- CO_2 at 16 MPa, a S:FR of 75, with a 240 cm column, could enrich an SBO DD containing ca. 10–15% tocopherols to ca. 50% tocopherols with a yield of ca. 80% [10] and SC- CO_2 at 18 MPa, a S:FR of 51, with a 300 cm column, could enrich an olive oil DD containing ca. 52% squalene to ca. 92% squalene with a yield of ca. 93% [11].

This research demonstrates that L- CO_2 can be used in a continuous counter-current fractionation system to effectively remove both unreacted EF and by-product FAEs from a mixture of TAGs, EF and FAEs. The purity of the TAG product increased with both the fractionation column length as well as the S:FR. The total flow within the system could also be increased to increase the throughput without adversely affecting the purity of the raffinate product. The close similarities between the EF:FAE:TAG model mixture investigated here and actual crude SoyScreenTM mixture (i.e., EF:FAE:TAG:FAG) suggests that this fractionation technique holds promise as an effective method to purify the crude SoyScreenTM enzymatic mixture. The significant process variables determined in this study should be directly applicable as well. Although this research did not utilize a reduced pressure receiver for collecting the extract and recycling the CO_2 , in the future, the system will be modified to utilize recycled CO_2 .

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